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Susceptibility of Inbred Mice to Rickettsiae of the Spotted Fever Group

CHRISTINE S. EISEMANN,* MATTHEW J. NYPAVER, AND JOSEPH V. OSTERMAN

Department of Rickettsial Diseases, Walter Reed Army Institute of Research, Washington, DC 20307

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A mouse strain susceptible to lethal infection with *Rickettsia conorii* was required for testing vaccine efficacy and for studying the immunology and pathogenesis of infection. Among 20 strains of inbred mice inoculated intraperitoneally with the Malish strain of *R. conorii*, the C3H/HeJ mouse strain was the most susceptible, with a 50% lethal dose of approximately 10 PFU. Infection of all mouse strains resulted in a measurable antibody response; the highest titers correlated with the greatest degree of rickettsial replication as measured by plaque assay of infected spleen homogenates. Inoculation of C3H/HeJ mice with 5.0 $\log_{10}$ organisms of strain Malish by the subcutaneous route did not result in lethal infection. The Casablanca and Moroccan strains of *R. conorii* were not lethal for C3H/HeJ mice and, in addition, produced plaques in L-929 cells morphologically distinct from those produced by the Malish strain. The only other spotted fever group rickettsia tested which produced a lethal infection in C3H/HeJ mice was *Rickettsia sibirica*. Sublethal infection with any of the spotted fever rickettsiae tested protected against lethal infection with *R. conorii*. These data established a lethal challenge system for examining the protective efficacy of spotted fever immunogens and presented evidence of biological variation among strains of *R. conorii*.

Vaccine testing with spotted fever group rickettsiae has been carried out primarily in male guinea pigs. However, the signs of spotted fever infection in guinea pigs (fever, scrotal edema, and erythema) are variable, subject to external influences, and difficult to interpret or quantify. In addition, seroconversion has been demonstrated in guinea pigs inoculated with one-tenth the 50% guinea pig fever dose, indicating that the animals supported an active infection without a measurable fever response (16). These problems with the guinea pig model prompted us to search for a highly susceptible mouse strain that could be used for testing spotted fever vaccines.

In the past, outbred mice and some commonly used inbred mouse strains have been of limited value in studies of spotted fever rickettsiae because infection of mice either was not lethal or resulted in erratic mortality. Recent studies, however, have emphasized that at least three critical factors influence the establishment of lethal rickettsial infection in mice: the genetic background of the mouse, the strain of rickettsia, and the route of inoculation. In a survey of inbred mouse strains, the Gilliam strain of *Rickettsia tsutsugamushi*, which was formerly thought to be avirulent for mice, was lethal for at least nine strains of mice, and among all of the mouse strains studied, the 50% mouse lethal dose varied by as much as 6.0 $\log_{10}$ doses (5). A similar study of the response of inbred mice to the Kaplan strain of *Rickettsia akari* demonstrated variability of mouse susceptibility to this spotted fever group rickettsia and identified a number of mouse strains that would be useful models for studying *R. akari* infection (1). Mice susceptible to lethal infection with other spotted fever group rickettsiae have not yet been identified; however, the previous studies with *R. tsutsugamushi* and *R. akari* suggested to us that mouse models for the more virulent human pathogens (i.e., *Rickettsia rickettsii* and *Rickettsia conorii*) could be found. Additionally, since spotted fever group rickettsiae are antigenically diverse (17) and heterogeneous in terms of human virulence, it was reasonable to expect that mouse strains that were susceptible or resistant to one spotted fever group organism might not respond similarly to all rickettsiae of the spotted fever group.

In this study, we tested a number of inbred mouse strains for susceptibility to the Malish strain of *R. conorii*, the causative agent of boutonneuse fever, and found that the responses of some mouse strains were different from those previously established with *R. akari* (1). We identified one mouse strain that was susceptible to lethal *R. conorii* infection (C3H/HeJ) and that could be used as a model for testing the efficacy of experimental spotted fever vaccines. We also found other genetically related mouse strains (C3H/HeDub and C3H/RV) which were either intermediately susceptible or resistant to *R. conorii* infection and which could be used with susceptible mice in studies of the in vivo immunological events affecting the death or survival of the animals.

MATERIALS AND METHODS

Mice. Twenty inbred mouse strains were used in this study. Strains A/HeJ, A/J, AKR/J, BALB/cByJ, BALB/cJ, B10.D2/nSn, CBA/J, C3H/HeJ, C57BL/6J, C57L/J, DBA/1J, DBA/2J, P/J, RF/J, SEC/1ReJ, SJL/J, and SWR/J were obtained from the Jackson Memorial Laboratories, Bar Harbor, Maine. BALB/cDub, C3H/HeDub, and C3H/RV mice were purchased from Flow Laboratories, Dublin, Va. All mice were tested at 8 to 12 weeks of age and all except P/J mice were females.

Rickettsiae. The following organisms were used at the indicated passage levels: *R. conorii* Malish (egg 12), Casablanca (egg 40), and Moroccan (egg 287); *R. rickettsii* Sheila Smith (egg 17); *Rickettsia sibirica* 246 (egg 20); *Rickettsia australis* Phillip (egg 121). Seed suspensions were prepared by standard methods (21) from the yolk sacs of embryonated chicken eggs (Spafas, Inc., Norwich, Conn.), shell-frozen in dry ice–95% ethanol, and stored at −70°C. Rickettsiae were quantified by plaque assay in monolayers of irradiated L-929 cells as described by Oaks et al. (15), and titers were expressed as PFU. For one experiment, the Malish strain of

* Corresponding author.

R. conorii was exposed to 300,000 rad of gamma radiation (Gammacell 220; Atomic Energy of Canada, Ltd., Ottawa, Canada) to prepare nonreplicating rickettsiae (2).

Determination of mouse susceptibility to rickettsial infection. All strains of inbred mice were tested by intraperitoneal (i.p.) inoculation of *R. conorii* Malish. C3H/HeJ mice were also inoculated by the subcutaneous (s.c.) route with the Malish strain of *R. conorii* and i.p. with the Casablanca and Moroccan strains of *R. conorii*, *R. rickettsii*, *R. sibirica*, and *R. australis*. Rickettsial seed suspensions were diluted in cold Snyder I diluent (6) so that 0.2 ml of inoculum contained from 5.0 $\log_{10}$ PFU through less than 1 PFU (10-fold dilutions). Five mice were inoculated with each rickettsial dilution and observed for 28 days. At the time each experiment was performed, the rickettsial dose was verified by plaque assay of the mouse inoculum. The 50% mouse lethal dose (MLD_{50}) was calculated for each mouse strain by a previously described method (3) and for simplicity is presented as the $\log_{10}$ MLD_{50} (e.g., an MLD_{50} equivalent to $10^{2.7}$ PFU is expressed as a $\log_{10}$ MLD_{50} of 2.7). On day 28, all mice surviving rickettsial infection were bled from the right axillary artery, and sera were collected and stored at −40°C until assayed. In some experiments, C3H/HeJ mice surviving rickettsial infection were bled into heparin-rinsed Pasteur pipettes (heparin, 5,000 U per ml; Flow Laboratories) from the retroorbital sinus before challenge with 3.0 $\log_{10}$ PFU of *R. conorii* Malish. Antibody titers were determined for all sera or plasma by an indirect immunofluorescence assay (IFA) (18) with *R. conorii* Malish antigen and fluorescein-conjugated goat anti-mouse immunoglobulins (Cappel Laboratories, Cochranville, Pa.).

Assessment of in vivo replication of rickettsiae. C3H/HeJ, C3H/HeDub, and C3H/RV mice were inoculated i.p. with 2.4 $\log_{10}$ PFU of *R. conorii* Malish. At 4, 7, and 14 days postinoculation, three mice of each strain were bled into heparin-rinsed Pasteur pipettes from the retroorbital sinus, and the plasma was stored at −40°C until assayed. Mice were killed by cervical dislocation, and the spleens were removed aseptically, weighed, and homogenized in a Tenbrook ground glass grinder with sufficient Snyder I diluent to effect a 10% (wt/vol) spleen suspension. The rickettsial content of each spleen suspension was determined by plaque assay, and the average number of rickettsial PFU per spleen was calculated. The day 14 data for C3H/HeJ mice could not be obtained because the 2.4 $\log_{10}$ PFU challenge dose killed these mice approximately 9 days after i.p. inoculation.

RESULTS

Response of inbred mouse strains to infection with the Malish strain of *R. conorii*. Inbred strains of mice varied considerably in their ability to resist lethal infection with *R. conorii* Malish. The smallest number of rickettsial PFU capable of killing half of the mice inoculated is shown for each mouse strain in Table 1. The mouse strains clearly fall into three response categories. C3H/HeJ mice were most susceptible to *R. conorii* infection ($\log_{10}$ MLD_{50} of 1.1), and five mouse strains, CBA/J, C3H/HeDub, DBA/1J, DBA/2J, and SJL/J mice, were intermediately susceptible ($\log_{10}$ MLD_{50} values ranging from 2.7 to 3.7). Fourteen other strains exhibited no mortality, even at the highest dose of *R. conorii* inoculated ($\log_{10}$ MLD_{50} of ≥5.5). It was notable that each of the three C3H mouse strains studied was found in a different response category (Table 1).

Antibody responses of susceptible, intermediate, and resistant strains of inbred mice to *R. conorii* Malish. Antibody titers were determined by IFA on sera collected from all inbred mouse strains 28 days after inoculation of 2.0 $\log_{10}$ PFU (Table 1). The mouse strains are listed by their lethal response categories to facilitate comparison of their antibody responses. Although there is some fluctuation among antibody titers within each response category, the resistant mouse strains generally exhibited lower antibody levels than did the intermediate or susceptible strains. The mean IFA antibody titer for resistant mice was 160, whereas intermediately susceptible mice exhibited a mean titer of 640, and the one susceptible strain exhibited an antibody titer of 2,560. This latter titer was determined from the sera of some surviving mice inoculated with 10, rather than 100, PFU which killed all the susceptible mice.

Antibody responses to irradiated, non replicating *R. conorii*. Since the C3H subline presented a range of responses from susceptible to resistant in terms of lethal infection that correlated with greater to lesser antibody responses, the C3H/HeJ, C3H/HeDub, and C3H/RV mouse strains were chosen for further study. When the mice were inoculated with irradiated, nonreplicating *R. conorii* (orginally 5.0 $\log_{10}$ PFU), the antibody responses of all three C3H strains were low and, importantly, were identical to each other (Table 2). This is in contrast to the graded antibody titers produced in the same C3H strains when the mice were given only 10 PFU of viable *R. conorii*. These data suggest that the C3H strains were capable of mounting a humoral antibody response of similar potency to *R. conorii* antigens and that the heightened antibody titers of C3H/HeJ and C3H/HeDub mice after

TABLE 1. Susceptibility and antibody response of inbred mice to the Malish strain of *R. conorii* categorized by response to intraperitoneal infection

Mouse strain	Log_{10} MLD_{50}[a]	Antibody titer[b]
Resistant		
A/HeJ	4.7	80
A/J	≥5.5	160
AKR/J	≥5.5	160
BALB/cByJ	≥4.5	160
BALB/cDub	≥5.5	160
BALB/cJ	≥5.5	160
B10.D2/nSn	≥5.5	80
C3H/RV	≥4.5	160
C57BL/6J	≥5.5	160
C57L/J	≥5.5	160
P/J	≥5.5	80
RF/J	≥4.5	40
SEC/1ReJ	≥5.5	80
SWR/J	≥5.5	160
Intermediate		
CBA/J	3.7	1,280
C3H/HeDub	2.7	1,280
DBA/1J	3.7	2,560
DBA/2J	3.3	640
SJL/J	3.5	640
Susceptible		
C3H/HeJ	1.1	2,560

[a] PFU per 0.2 ml required to kill 50% of the inoculated animals.
[b] Reciprocal of the highest dilution of day 28 pooled sera reacting in an IFA. Serum was obtained after inoculation of 2.0 $\log_{10}$ PFU for all mouse strains except C3H/HeJ. Serum from C3H/HeJ mice was collected from animals surviving a 1.0 $\log_{10}$ PFU inoculum.

inoculation with viable organisms was due to an increased antigenic load resulting from rickettsial replication.

Replication of *R. conorii* in mouse strains of varying susceptibility. To determine whether replication of *R. conorii* was restricted in C3H/RV mice but not in C3H/HeJ or C3H/HeDub strains, mice were inoculated i.p. with 2.4 $\log_{10}$ PFU of *R. conorii* Malish. Spleens and sera were obtained at 4, 7, and if possible, 14 days postinoculation, and rickettsiae in the spleen cell homogenates were quantified by plaque assay. There was substantial and similar replication of rickettsiae during the first week of infection of C3H/HeJ and C3H/HeDub mice (Table 3). Four days after i.p. inoculation of 2.4 $\log_{10}$ PFU, the spleens of both mouse strains contained approximately 6.0 $\log_{10}$ PFU, and the number of rickettsiae per spleen rose to an apparent peak of 7.0 $\log_{10}$ PFU on the seventh day of infection. Susceptible C3H/HeJ mice succumbed to *R. conorii* infection approximately 9 days after inoculation, whereas C3H/HeDub mice were able to resolve the infection such that by the end of the second week, the numbers of rickettsiae in their spleens were at undetectable levels. At each time point studied, C3H/RV mouse spleens contained low or undetectable levels of *R. conorii* rickettsiae. Antibody titers began to rise by day 7 only in the two mouse strains in which replication of the organisms could be demonstrated (Table 3). By the end of the second week, *R. conorii* antibodies in C3H/HeDub and C3H/RV mice had reached maximum levels and showed a difference in IFA titers similar to that previously demonstrated with sera from day 28.

Effect of route of inoculation on susceptibility to infection. Previous studies with *R. tsutsugamushi* showed that i.p. inoculation of Gilliam strain rickettsiae into susceptible mice resulted in lethal infection, but if the mice were given the same inoculum s.c., a sublethal infection was established and the mice survived (5). Conversely, Anderson and Osterman (1) demonstrated that infection of C3H/HeJ mice with *R. akari* Kaplan was lethal regardless of which inoculation route was used. Therefore, duplicate titrations with the Malish strain of *R. conorii* were performed both i.p. and s.c. in C3H/HeJ mice. The $\log_{10}$ MLD_{50} values are shown in Table 4. Although C3H/HeJ mice were susceptible to low challenge doses of Malish strain organisms inoculated i.p., they completely resisted s.c. challenge of 5.0 $\log_{10}$ PFU. Additionally, C3H/HeJ mice inoculated s.c. responded with low antibody titers which were similar in magnitude to those developed in inbred mouse strains resistant to i.p. challenge. C3H/HeJ mice surviving 28 days after s.c. inoculation of 3.0 to 5.0 $\log_{10}$ PFU Malish strain rickettsiae were immune from an otherwise lethal i.p. inoculation of 3.0 $\log_{10}$ PFU of the same organisms.

Susceptibility of C3H/HeJ strain mice to other spotted fever group rickettsiae and various strains of *R. conorii*. The susceptibility of C3H/HeJ mice to lethal infection with the Malish strain of *R. conorii* and to one strain of *R. akari* (1) suggested that other spotted fever group rickettsiae might be similarly lethal for C3H/HeJ mice. Additionally, variation between the apparent virulence of the Kaplan and Hartford strains of *R. akari* for C3H/HeJ mice (1) suggested that other strains of *R. conorii* be tested for lethality in these animals. Therefore, C3H/HeJ mice were inoculated i.p. with the Casablanca and Moroccan strains of *R. conorii* as well as with *R. rickettsii*, *R. sibirica*, and *R. australis*. After 28 days, mice which had received the maximum rickettsial dose (5.0 $\log_{10}$ PFU) were bled from the retroorbital sinus and then challenged i.p. with a lethal dose of the Malish strain of *R. conorii*. Only *R. sibirica*, with a $\log_{10}$ MLD_{50} of 0.7, demonstrated a virulence for C3H/HeJ mice similar to that of Malish strain rickettsiae (Table 5). It was notable that neither the Casablanca nor the Moroccan strain of *R. conorii* established a lethal infection in C3H/HeJ mice. In all cases, plasma collected from mice surviving the initial rickettsial infection reacted in an IFA with the *R. conorii* Malish antigen. Heterologous antibody titers of 160 to 320 indicated that rickettsial infection was established in these animals. In addition, sublethal infection with any of the spotted fever rickettsiae studied protected C3H/HeJ mice from lethal challenge with 3.0 $\log_{10}$ PFU of *R. conorii* Malish.

Plaque morphologies of *R. conorii* strains. In addition to the observed virulence for C3H/HeJ mice, the Malish strain of *R. conorii* produced plaques in irradiated L-929 cells which differed in morphology from those produced by the other *R. conorii* strains (Fig. 1). The Casablanca and Moroccan strains both produced clear plaques (diameter, 1 mm) which were devoid of any viable cells within the perimeter of the plaque. Malish strain plaques however, although the same

TABLE 2. Antibody response of C3H mouse strains to irradiated and nonirradiated *R. conorii* Malish

Mouse strain	Antibody titer[a]	
	5.0 $\log_{10}$ PFU irradiated[b]	1.0 $\log_{10}$ PFU nonirradiated
C3H/HeJ	80	2,560
C3H/HeDub	80	1,280
C3H/RV	80	160

[a] Reciprocal of the highest dilution of day 28 pooled sera reacting in an IFA.
[b] 300,000 rad gamma radiation.

TABLE 3. PFU of Malish strain rickettsiae in homogenates of spleens from C3H mice at indicated times after inoculation of 2.4 $\log_{10}$ PFU

Mouse strain	$\log_{10}$ PFU per spleen		
	Day 4	Day 7	Day 14
C3H/HeJ	6.3 (<20)[a]	7.5 (80)	ND[b]
C3H/HeDub	5.9 (<20)	7.1 (20)	<2.0 (1,280)
C3H/RV	<2.0 (<20)[c]	<2.0 (<20)	<2.0 (320)

[a] Reciprocal of the highest dilution of day 28 pooled sera reacting in an IFA.
[b] ND; not determined. C3H/HeJ mice died approximately 9 days after inoculation.
[c] Fewer than 100 PFU per spleen were undetectable in these experiments.

TABLE 4. Effect of the route of inoculation on the susceptibility of C3H/HeJ mice to infection with *R. conorii* Malish

Inoculation route	$\log_{10}$ MLD_{50}[a]	Antibody titer[b]	Deaths/mice reinoculated[c]
i.p.	1.1	2,560	not done
s.c.	5.1	320	0/8

[a] PFU per 0.2 ml required to kill 50% of the inoculated animals.
[b] Reciprocal of the highest dilution of day 28 pooled plasma reacting in an IFA. Plasma was collected from mice surviving the highest rickettsial dose, i.e. 1.0 $\log_{10}$ PFU inoculated i.p. and 5.0 $\log_{10}$ PFU inoculated s.c.
[c] Mice surviving 28 days after s.c. inoculation of 5.0 $\log_{50}$ PFU of Malish strain rickettsiae were reinoculated i.p. with 3.0 $\log_{10}$ PFU of the same organisms.

TABLE 5. Susceptibility of C3H/HeJ mice to lethal infection with *R. conorii* strains and other spotted fever group rickettsiae

Rickettsia	Log_{10} MLD_{50}[a]	Antibody titer[b]	Deaths/mice reinoculated[c]
R. conorii			
Malish	1.1	ND[d]	ND
Casablanca	5.3	320	0/9
Moroccan	5.1	160	0/5
R. australis	5.3	160	0/9
R. rickettsii	≥5.5	160	0/5
R. sibirica	0.7	ND	ND

[a] PFU per 0.2 ml required to kill 50% of the inoculated animals.
[b] Reciprocal of the highest dilution of day 28 pooled plasma reacting with *R. conorii* Malish antigen in an IFA. Plasma was collected from mice surviving a 5.0 log_{10} PFU rickettsial dose.
[c] Mice surviving 28 days after i.p. inoculation of 5.0 log_{10} PFU rickettsiae were reinoculated i.p. with 3.0 log_{10} PFU of *R. conorii* Malish.
[d] ND, Not done.

size as those of the other two strains, contained viable cells in the center of the plaque which absorbed the neutral red vital stain.

DISCUSSION

We evaluated 20 strains of inbred mice for susceptibility to the Malish strain of *R. conorii* to identify which mouse strain(s) could be used as a model for studying infection with this spotted fever group rickettsia. The C3H/HeJ mouse strain was the most useful model, as titration of *R. conorii* in these animals resulted in consistently lethal dose-response patterns, and the mice were susceptible to lethal infection with a small number of rickettsiae (only 1.1 log_{10} PFU of *R. conorii* was required to kill 50% of the C3H/HeJ mice tested). Other mouse strains were at least 100-fold less susceptible to lethal *R. conorii* infection than were C3H/HeJ mice, including the DBA/2J strain which has been used in other studies of *R. conorii* infection (12, 19, 20). In our experiments, mice inoculated i.p. with 2.4 log_{10} PFU of *R. conorii* rickettsiae (approximately 10 MLD_{50}) consistently died 8 to 9 days after inoculation. During the infection, rickettsial titers in the spleens of these animals rose to 7.5 log_{10} PFU per spleen 7 days after inoculation, despite the presence of moderate levels of antibody detectable in the serum. The production of *R. conorii* antibody by these mice and the fact that sublethal infection with other spotted fever group rickettsiae protected them from lethal *R. conorii* infection indicate that C3H/HeJ mice are immunologically competent in terms of the ability to respond to spotted fever antigens.

The C3H/HeJ mouse strain was unique among the C3H strains studied in the degree of susceptibility to infection with *R. conorii* Malish. C3H/HeDub mice were intermediately susceptible, and although infection of these animals resulted in rickettsial replication similar to that seen in susceptible C3H/HeJ mice, C3H/HeDub animals were able to resolve the infection and survive. C3H/RV mice were resistant to even the highest dose of *R. conorii* injected (5.0 log_{10} PFU) and, apparently, were able to restrict in vivo rickettsial replication. Since it has been demonstrated for other rickettsiae that cell cultures derived both from susceptible and resistant mouse strains support rickettsial growth (1, 5), the difference in *R. conorii* replication between C3H/RV mice and the other C3H mouse strains probably reflected variation in the immune reponses mounted by these animals during rickettsial infection. The high antibody levels observed in susceptible mice apparently resulted from the increased immunogenic load caused by rickettsial replication. Immunization of mice with a high dose (5.0 log_{10} PFU) of nonreplicating rickettsiae resulted in low antibody levels as compared with antibody obtained after inoculation of susceptible mouse strains with a substantially lower dose (1.0 log_{10} PFU) of viable rickettsiae. Importantly, and as previously demonstrated with other rickettsial infections in mice (7, 11), the presence of high levels of antibody did not affect the ultimate survival of the animals. It should be noted, however, that antibody titers were measured with intact rickettsiae, and it is possible that the mouse strains responded differently to individual rickettsial components.

Spotted fever group rickettsiae clearly differed in lethality for C3H/HeJ mice. Strains of *R. akari* (1), *R. conorii*, and *R. sibirica* were highly lethal for these animals, whereas *R.*

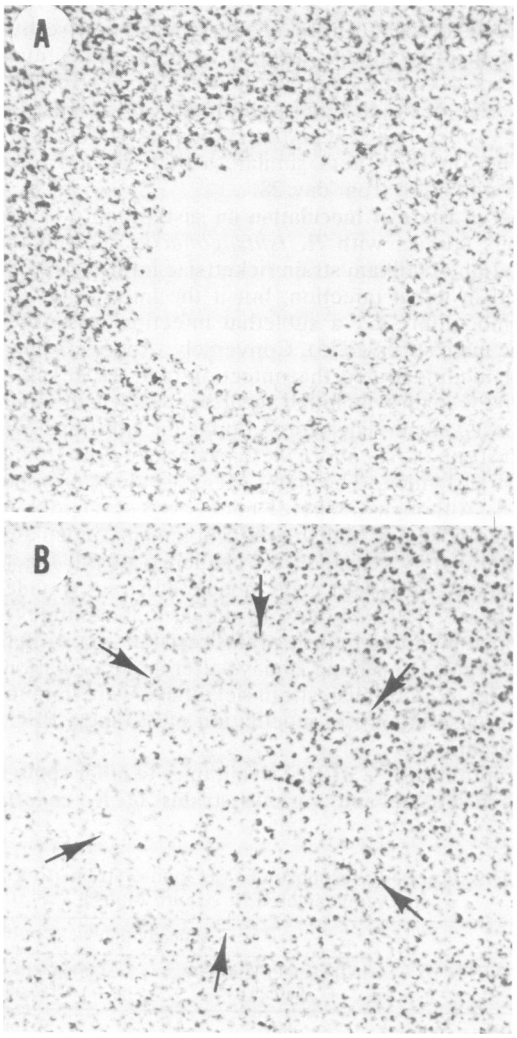

FIG. 1. Plaques formed by strains of *R. conorii* in irradiated L-929 cells. (A) Clear plaque produced by the Casablanca strain. The Moroccan strain produced similar clear plaques. (B) Target-type plaque produced by the Malish strain. The arrows delineate the perimeter of the plaque.

rickettsii and *R. australis* did not establish lethal infections even when infectious doses of 5.0 $\log_{10}$ PFU were given. The virulence of *R. conorii* and *R. sibirica* paralleled the results of a previous study (16) in which the infectivity of these two rickettsiae in Swiss mice were 100 to 1,000 times greater than that observed with *R. rickettsii*. In contrast to *R. akari* infection (1), the route of inoculation of C3H/HeJ mice with the Malish strain of *R. conorii* critically affected the outcome of the infection: i.p. inoculation of Malish strain organisms established a lethal infection, but if the mice were given a similar inoculum s.c., the mice survived. Sublethal infection of C3H/HeJ mice with any of the spotted fever group rickettsiae tested resulted in protection of the animals from otherwise lethal challenge.

Of the three *R. conorii* strains used in this study, only the Malish strain caused a lethal infection of C3H/HeJ mice. Casablanca and Moroccan rickettsiae established sublethal infections but did not kill the animals at any dose tested. Selection of the Malish strain for the initial screening of inbred mice was based on the observation that this strain of *R. conorii* caused a greater fever response in guinea pigs (16) than was usually obtained with the Casablanca strain. Variation in C3H/HeJ lethality after infection with strains of *R. akari* has also been reported (1). The differences we observed in the plaque morphologies of the three *R. conorii* strains was an interesting correlate to their lethality for C3H/HeJ mice and suggested a formerly unidentified basis of strain differentiation for these spotted fever organisms. Plaque morphology, however, did not correlate with virulence of spotted fever rickettsiae for C3H/HeJ mice. The clear plaques obtained in irradiated L-929 cells with the Casablanca and Moroccan strains of *R. conorii* are unusual for spotted fever group rickettsiae. Under the same plaquing condition, all other spotted fever rickettsiae tested, including C3H/HeJ-virulent and -avirulent rickettsiae, produced the target-type plaques previously described for spotted fever rickettsiae (9, 22, 23). The variable characteristics we observed among the Malish, Casablanca, and Moroccan strains of *R. conorii*, although possibly affected by strain passage level, also may reflect strain variation based on geographical isolation, a factor which has been shown to influence the severity of *R. conorii* infections in humans (4).

We have demonstrated in this study that lethal infection of C3H/HeJ mice could not be established by all spotted fever group rickettsiae and not by all strains within one rickettsial species. This observation is consistent with results obtained after infection of mice with several strains of *R. akari* (1) and contributes to the evolving concept that rickettsial infection in mice is a complex event that depends on the genetic background of the mouse, as well as the strain of rickettsiae used and the route of inoculation into the animal host. In addition, it appears that the immunological events which modulate a rickettsia-host interaction may not be generally characteristic of all rickettsial infections. For example, infection of BALB/c mice with *Rickettsia typhi*, a typhus group rickettsia, has been shown not to be correlated with the microbicidal activity of activated macrophages (A. E. Christ, Jr., and C. L. Wissemann, Jr., Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, E46, p. 58); yet, other studies of rickettsial infection in mice (13, 14) indicate that functional macrophages are necessary for mouse survival. In a study of *R. akari* infection, Anderson and Osterman (1) showed that, in addition to C3H/HeJ mice, mouse strains derived from A strain mice (A/HeJ, AWySn, and A/J) were among the most susceptible to *R. akari* infection. Later, Meltzer and Nacy (13) linked the rickettsial susceptibility of A strain mice and other inbred mouse strains to defects in macrophage function. In contrast to results obtained with *R. akari*, our study showed that A strain mice were markedly resistant to lethal infection with the Malish strain of *R. conorii* and suggested that the A strain macrophage defect does not affect mouse susceptibility to this spotted fever group rickettsia. In addition, two mouse strains which are defective in macrophage tumoricidal activity, the lipopolysaccharide-unresponsive C3H/HeJ strain and P/J mice (14), demonstrated different susceptibilities to infection with *R. conorii* Malish. Thus, the impact of macrophage defects on the pathogenesis of infection in mice may vary among spotted fever group rickettsiae and perhaps among the other rickettsiae as well.

The C3H/HeJ mouse strain is a useful animal model for studying *R. conorii* infection in that the evaluation of infection or protection from challenge clearly is objective, i.e., the animals either live or die. Lethal infection is established in this mouse strain with lower doses of *R. conorii* rickettsiae than those used in other studies with different inbred mouse strains (12, 19, 20) and, in addition, is established without treating the animals with cyclophosphamide, a drug which has been used to enhance rickettsial infection in mice (10, 12). Importantly, C3H/HeJ mice can be immunized against lethal *R. conorii* infection. Additionally, the identification of other C3H mouse strains (C3H/HeDub and C3H/RV) of different susceptibilities to *R. conorii* infection makes it possible to compare mechanisms of susceptibility or resistance as has been done with other rickettsiae, i.e., activation of inbred mouse macrophages (14), macrophage defects (13), and the nature of the inflammatory cell response (8), which have all been studied for their influence on mouse survival after rickettsial infection.

Thus, the C3H/HeJ mouse strain constitutes an excellent animal model for studying the pathogenesis of *R. conorii* infection and for testing the immunogenic potential of experimental rickettsial vaccines.

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